

VI. Specification Amendments under 37 C.F.R. § 1.121(b)

1) On page 14 of the instant specification, please replace the paragraph that begins with the following words, "Nucleic acid molecules having at least 10 nucleotides and exhibiting sequence..." with the following replacement paragraph:

Nucleic acid molecules having at least 10 nucleotides and exhibiting sequence complementarity or homology to the proto-oncogenes described herein find utility as hybridization probes. It is known in the art that a "perfectly matched" probe is not needed for a specific hybridization. Minor changes in probe sequence achieved by substitution, deletion or insertion of a small number of bases do not affect the hybridization specificity. In general, as much as 20% base-pair mismatch (when optionally aligned) can be tolerated. Preferably, a probe useful for detecting the aforementioned proto-oncogene mRNA is at least about 80% identical to the homologous region of comparable size contained in the previously identified sequences, which have the GenBank® accession number identified in Table 2 (below). More preferably, the probe is 85% identical to the corresponding gene sequence after alignment of the homologous region; even more preferably, it exhibits 90% identity. Specifically, a preferred probe for b-myb is TGCTGCCCTG (SEQ ID NO. 1), a preferred probe for PGP9.5 is CAGTCTAAAA (SEQ ID NO. 2), a preferred probe for 8-oxo-dGTPase is TGGCCCGACG (SEQ ID NO. 3), and a preferred probe for p67 is TAATACTTTT (SEQ ID NO. 4), or their respective complements. Additional probes can be derived from sequences for these genes identified by the GenBank® Accession numbers provided in Table 2 or to a homologous region of comparable size contained in the previously identified sequences, which have the GenBank® accession numbers identified in Table 2. These probes can be used in radioassays (e.g. Southern and Northern blot analysis) to detect, prognose, diagnose or monitor various neoplastic states resulting from the overexpression of PGP9.5, p67, 8-oxo-dGTPase or the b-myb genes. The total size of the fragment, as well as the size of the complementary stretches, will depend on the intended use or application of the particular nucleic acid segment. Smaller fragments derived from the known sequences will generally find use in hybridization embodiments, wherein the length of the complementary region may be varied, such as between about 10 and about 100 nucleotides, or even full length according to the complementary sequences one wishes to detect.

2) On page 18 of the instant specification, please replace the paragraph that begins with the following words, "Detectable labels suitable for use in the present invention..." with the following replacement paragraph:

Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads (e.g., Dynabeads[[TM]]®), fluorescent dyes (e.g., fluorescein, Texas Red®, rhodamine, green fluorescent protein, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P) enzymes (e.g., horse radish peroxidase, alkaline phosphatase and other commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Patents Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241).

3) On page 22 of the instant specification, please replace the paragraph that begins with the following words, "When the agent is a nucleic acid, it can be added to the cell cultures..." with the following replacement paragraph:

When the agent is a nucleic acid, it can be added to the cell cultures by methods well known in the art, which includes, but is not limited to calcium phosphate precipitation, microinjection or electroporation. Alternatively or additionally, the nucleic acid can be incorporated into an expression or insertion vector for incorporation into the cells. Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from sources such as Stratagene® (LaJolla, CA) and Promega Biotech® (Madison, WI). In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add, or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression. Examples of vectors are viruses, such as baculovirus and retrovirus, bacteriophage, adenovirus, adeno-associated virus, cosmid, plasmid, fungal vectors, and other recombinant vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

4) On page 31 of the instant specification, please replace the paragraph that begins with the following words, "The sequence and occurrence of each of the transcript tags was with the following replacement paragraph:

The sequence and occurrence of each of the transcript tags was determined using SAGE software, described, for example in Venter *et al.* (1996) *Nature* 381: 364-366. To identify transcript tags present in each library, the sequence of all SAGE tags were stored as "tag" file in Microsoft Access®. The GenBank® dbEST and nucleotide databases were also analyzed by the SAGE software to identify the corresponding SAGE tags and then stored as a "Genename." The GenBank® entry for each SAGE tag was obtained by linking the tags from Tag and Genename files using Microsoft Access®. The relative occurrence of each tag was determined by comparing the number of tags observed in the tumor libraries with that observed in the normal control libraries. The relative abundance for the tags was calculated by dividing the total number of tags observed with the total number of tags identified.

5) On page 32 of the instant specification, please replace the paragraph that begins with the following words, "Cell lysates from 5×10^4 cells of each cell line described herein were...", with the following replacement paragraph:

Cell lysates from 5×10^4 cells of each cell line described herein were electrophoresed on a 4-20% SDS gradient gel and transferred to a PVDF membrane (MSI). After blocking non-specific sites by incubating in PBS + 5% non-fat dry milk (NFDM), the membrane was incubated with anti-PGP9.5 antibody (Biogenesis, UK) at 1:400 dilution. ECL kit (Amersham®) was used to visualize the antibody binding to PGP9.5 protein (see Fig 3B bottom panel).

6) On page 32 of the instant specification, please replace the paragraph that begins with the following words, "Four independent SAGE libraries were constructed from messenger RNAs using..." with the following replacement paragraph:

Four independent SAGE libraries were constructed from messenger RNAs using two squamous cell lung cancers and two normal lung small airway epithelial cell cultures as described in Madden *et al.* (1997) *Oncogene* 15:1079-1085. A total of 2,000-4,000 clones were sequenced to identify more than 50,000 transcript tags from each library (Table 1). The sequences of over 50,000 tags that represent about 15,000 unique transcripts in each library were analyzed in order to generate a comprehensive profile of gene expression patterns in lung cancer. In total, 226,876 tags were sequenced, jointly representing 43,254 unique transcripts. GenBank® analysis

suggested that about 40% of the SAGE tags had at least one match in the database. As summarized in Table 1, an examination of the SAGE tags identified from each sample indicated that the occurrence of tags within each tissue type was highly consistent because only 15 and 17 tags were differentially expressed by more than 10 fold when the two normal control libraries were compared with each other. Similarly, 36 and 39 tags, respectively, were expressed differentially by more than 10-fold between the two tumors. Therefore, the SAGE tags obtained from the two normal controls and the two tumors were combined to determine[[d]] the total number of occurrence for each tag.

7) On page 33 of the instant specification, please replace the paragraph that begins with the following words, "All tags were searched against GenBank to identify the corresponding gene transcripts..." with the following replacement paragraph:

All tags were searched against GenBank® to identify the corresponding gene transcripts or EST clones. The highest level of relative gene expression in the normal control was ~~about~~ about 150 fold (data not shown), whereas the highest level of expression in the tumor was 57 fold compared to the normal control.

8) On page 33 of the instant specification, please replace the paragraph that begins with the following words, "The transcripts to 15 significantly overexpressed tags with authentic EST..." with the following replacement paragraph:

The transcripts to 15 significantly overexpressed tags with authentic EST or GenBank® matches have been examined. Among the tested genes, 10 were excluded from further analysis because they were either commonly expressed (6 genes) or virtually absent (4 genes) in most tissues and cell lines. (See, Table 2). Interferon- α -inducible gene was detected in the normal lung and therefore was not further analyzed. The transcripts for four genes, each encoding 8-oxo-dGTPase (human *MutT*), b-myb, p67 or PGP9.5, were not detectable in the normal lung samples but were easily detectable in most of the lung cancer cell lines tested (Figs. 2 and 3B). The abundance of these four genes ranged [[form]] from 0.012% to 0.018% of the total tags identified in the tumor or ~~about~~ about 36-54 transcripts/cell, assuming that there are approximately 300,000 transcripts in a cell.

VII. Conclusion

No fee is deemed necessary in connection with the filing of this communication. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 07-1074.

3/21/05

Date

Respectfully submitted,



Jennifer D. Tousignant
Agent for Applicants
Registration No. 54,498
Telephone: (508) 270-2499
Facsimile: (508) 872-5415

GENZYME CORPORATION
15 Pleasant Street Connector
P.O. Box 9322
Framingham, Massachusetts 01701-9322